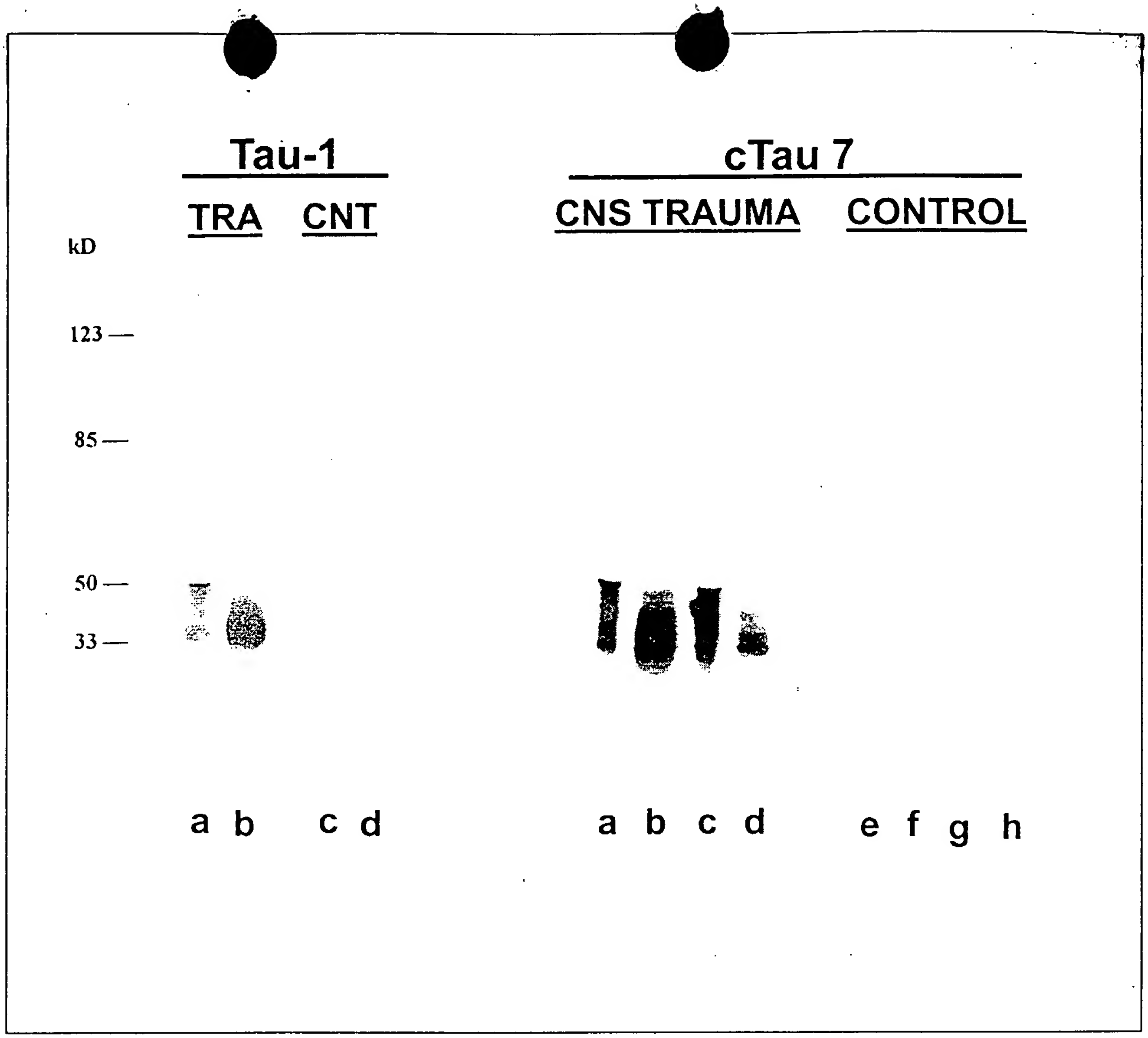

Fig. 1. Monoclonal antibodies recognizing cerebrospinal fluid cleaved tau proteins were developed by differential CSF screening. *Left panel:* Initial studies demonstrated that Mab Tau-1 labeled 30 kDa to 50 kDa CSF proteins in CNS trauma patients (TRA; lane a 50 μl CSF, lane b 10 μl CSF) but not normal controls (CNT; lane c and d both 50 μl CSF). *Right panel:* Mabs were developed that specifically recognized 30 kDa to 50 kDa CSF tau proteins employing a differential CSF screen. Hybridomas were selected that labeled CSF from CNS trauma patients (lanes a-d) but not control patients (lanes e-h). The same patient samples were run in lanes a and b in left and right panels. cTau7 lanes a and e-h 50 μl CSF, lanes b-d 10 μl CSF. Molecular weight markers shown at left.

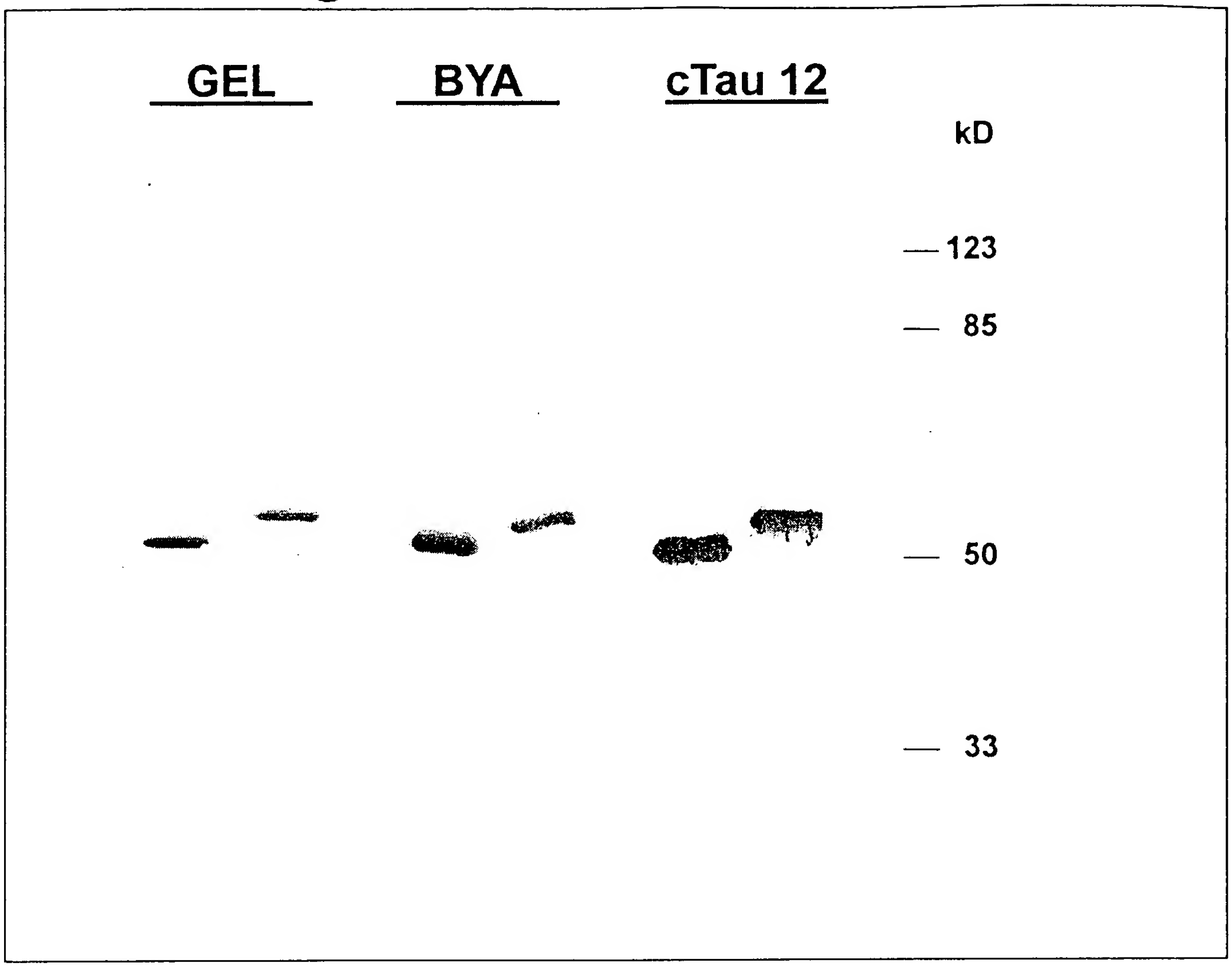

Fig. 2. CSF cleaved tau Mabs label recombinant tau. Commassie Blue stained 10% gel with recombinant 3-repeat tau (Gel, left lane, 1 μg) and 4-repeat tau (Gel, right lane, 1 μg). Recombinant tau blotted with BYA (0.07 μg/lane) and cTau12 (2.5 μg/lane). Molecular weight markers shown at right.

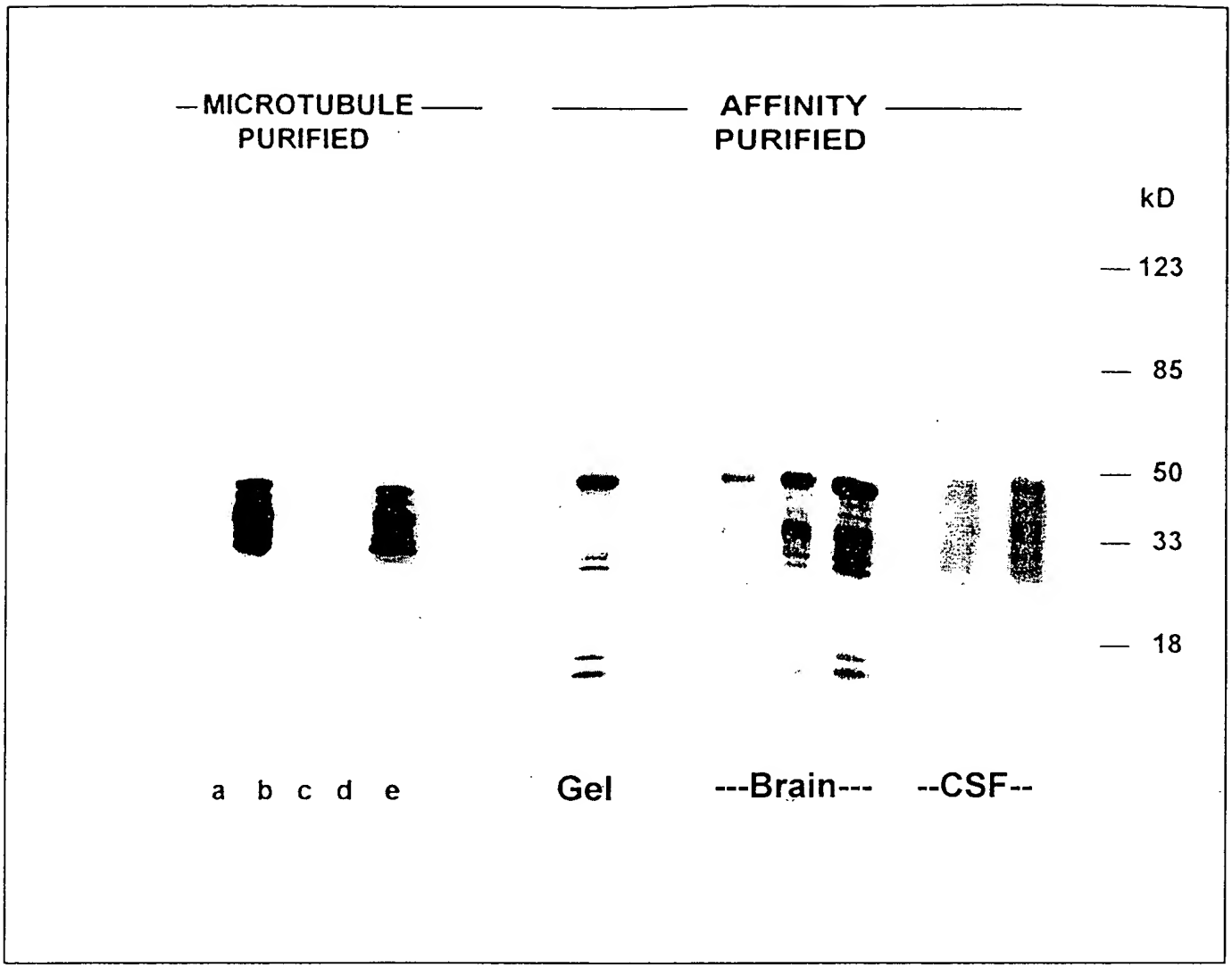

Fig. 3. CSF and brain cleaved tau proteins bind microtubules. *Microtubule Purified:* Initially, taxol polymerized microtubules were salt extracted to insure that no cTau7 immunoreactive proteins were present (lane a). Microtubules were then incubated with a preparation of CSF cleaved tau proteins (lane b, 1 μg) and washed several times until the supernatant was free of cTau7 reactivity (lanes c and d). Microtubule bound proteins were salt extracted yielding 30 kDa to 50 kDa cTau7-reactive cleaved tau proteins (lane e). Similar results were obtained with Mabs cTau8 and cTau12 (data not shown). *Affinity Purified:* cleaved tau proteins were affinity purified from either CSF or brain with Mabs cTau7, cTau8 and cTau12 coupled to Protein G agarose. Commassie Blue stained gels indicated that affinity purified cleaved tau consisted of a primary 50 kDa protein band (gel, 1 μg). Immunoblots of affinity purified CSF (100 and 500 ng) and brain (10, 30 and 100 ng) with cTau7 revealed 30 kDa to 50 kDa protein bands. Similar results were observed with Mabs cTau8 and cTau12 (data not shown). Position of molecular weight markers shown at right.

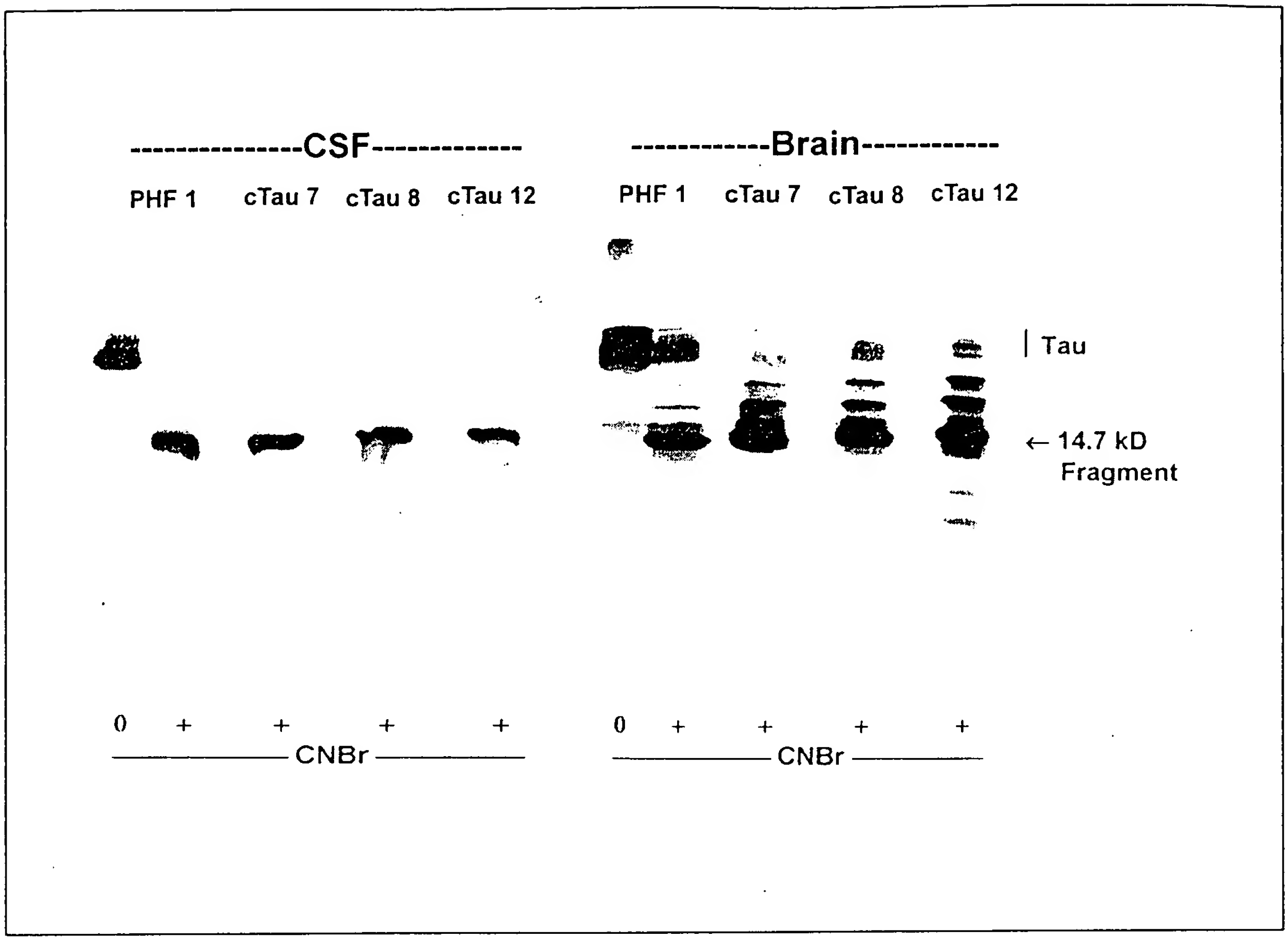

Fig. 4. Mabs cTau7, cTau8 and cTau12 recognize a 14.7 kDa CNBr digestion fragment occurring in patient CSF or brain. Cleaved tau from CSF or brain (2 μg/lane) was treated either with (+) or without (0) CNBr and blotted with Mabs PHF-1, cTau7, cTau8 and cTau12. All four antibodies appeared to predominantly label the same CNBr digestion fragment consisting of tau amino acids pro^{251} to met^{419} . The PHF-1 fragment has a reported molecular weight of 14.7 kDa (Zemlan and Dean, 1996). Blots are from a single 15% gel. The position at which non-digested cleaved tau migrated is shown (Tau).

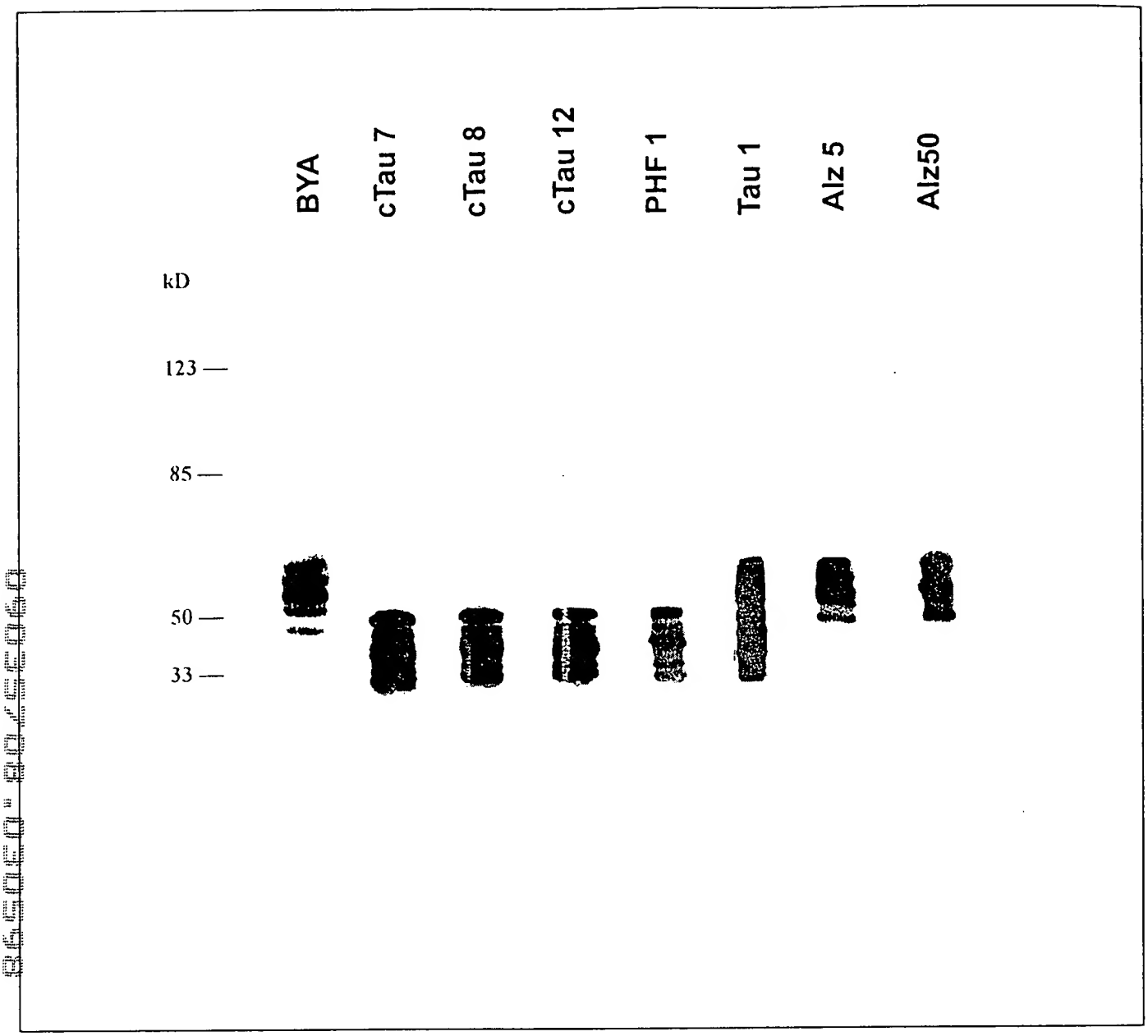

Fig. 5. *Post Mortem* brain contains both full length 48 kDa to 68 kDa tau proteins and 30 kDa to 50 kDa cleaved tau proteins. Intact tau proteins were selectively labeled with the tau antibody BYA, the C-terminal tau antibody Alz5 and the N-terminal tau antibody Alz50 in heat stable preparations of *post mortem* brain. Cleaved tau proteins were selectively labeled with Mabs cTau7, cTau8, cTau12 and PHF1 that recognizes phospho-ser^{396} of tau. Mab Tau-1 that recognizes non-phosphorylated ser^{199} of tau labeled both forms of tau. These data suggest that tau is cleaved and phosphorylated at ser^{396} in brain.

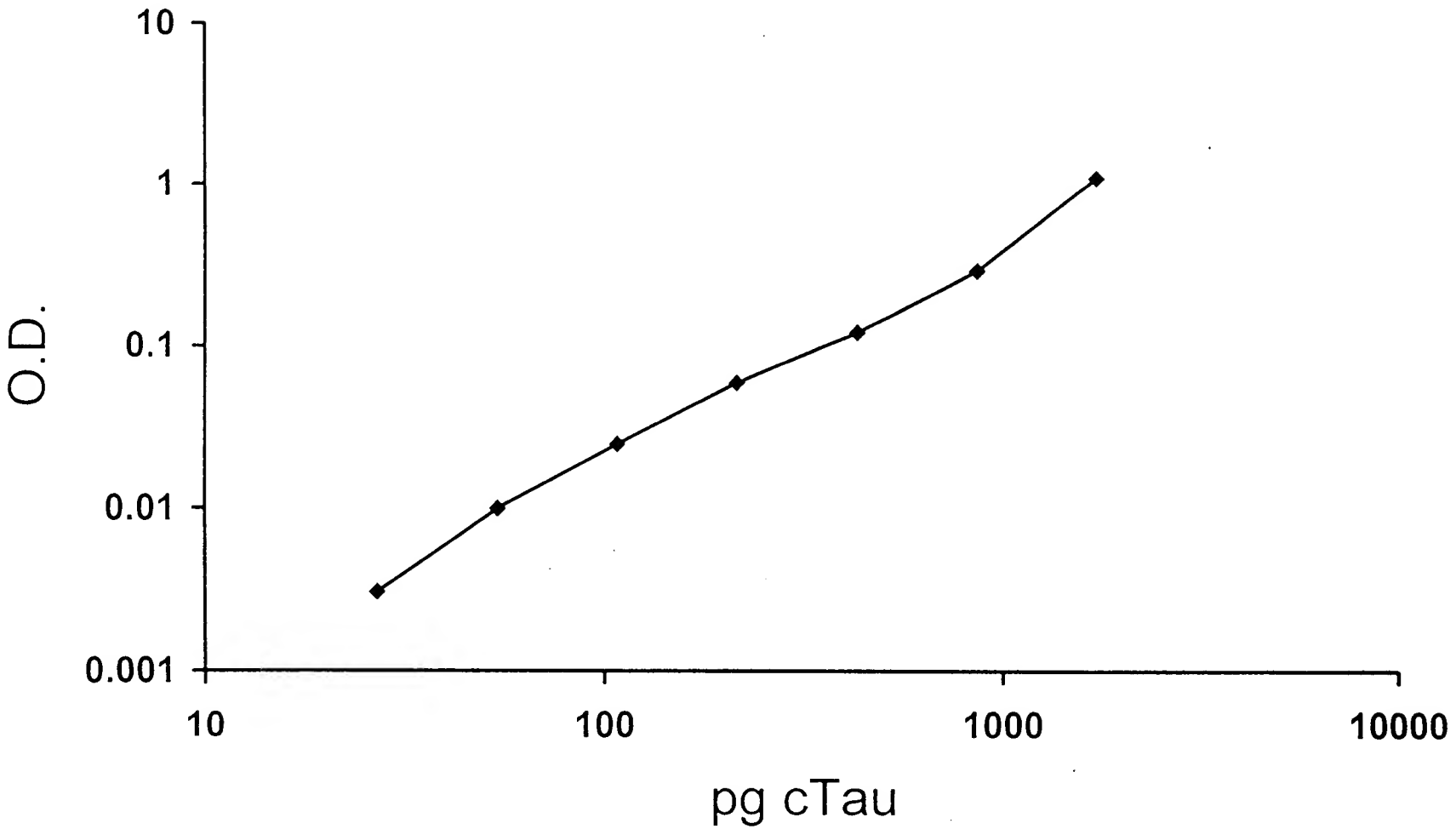

Fig. 6. Titration of affinity purified CSF cleaved tau employing the developed cleaved tau ELISA. A catalyzed-reporter deposition sandwich ELISA was developed employing cTau12 as caputure antibody and HRP-conjugated cTau7 and cTau8 for decetion. Affinity purified CSF cleaved tau was used as standard. All concentrations (pg/well) were tested in triplicate and values represent the mean O.D. value.